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Sulfenamides as prodrugs of NH-acidic compounds: A new prodrug option for the amide bond

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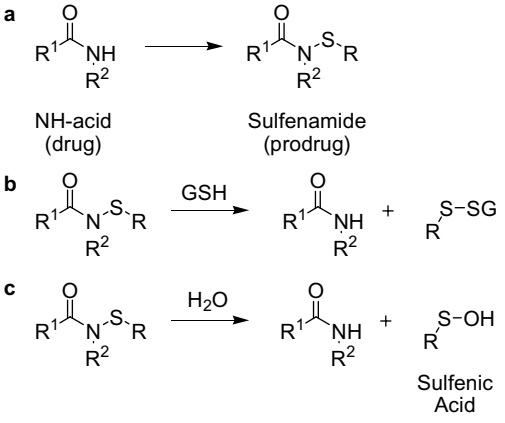
Abstract—The objective of this report is to introduce the novel concept of utilizing sulfenamides as prodrugs for compounds containing an NH-acidic functionality, particularly weakly acidic amide-type functionalities (amides, ureas, carbamates, etc.). Included are the syntheses and physicochemical characterizations of some model sulfenamides to illustrate the promise of this new prodrug technology.

A continual challenge in Drug Discovery is balancing the time dedicated to lead optimization against the ultimate goal of quickly identifying a suitable candidate for development. Prodrugs[1,2] can reduce this lead optimization time, particularly when a discovery team has identified a molecule that meets most of the team's selection criteria, but presents a significant obstacle to drug delivery due to limitations in properties such as solubility, dissolution rate, permeability, stability, etc. A well-designed prodrug is a non-toxic, chemically modified derivative of the drug that is easier to deliver; but once delivered, will reconvert, generally in a rapid and quantitative fashion, to the drug so that it can exert its therapeutic action. Designing a well-behaved prodrug can be a challenging task, so the more viable options that are available to the medicinal chemist, the greater the chance for success. Despite the number of prodrug approaches previously attempted for NH-acids,[3] none have provided a reliable and generic way of making prodrugs that are hydrolytically stable, but rapidly and quantitatively bioreversible; more specifically, the void in synthetic options is particularly significant for the weakly acidic NH-acids, such as amides, ureas, carbamates, etc. ($pK_a > 12$–13).[3,4] The objective of this report is to introduce a new option which utilizes sulfenamides as prodrugs of NH-acids (Scheme 1a). And while we have evaluated sulfenamides of NH-acids possessing a wide range of acidities,[5] this report will focus solely on sulfenamides of weakly acidic NH-acids, since that is the sub-group in greatest need of new prodrug ideas.

A sulfenamide[6] is identified by a N–S single bond where the sulfur atom is bivalent. This single N–S bond is typically polarized with the partial positive charge residing on the sulfur atom. The sulfur atom is susceptible to nucleophilic attack resulting in a displacement reaction that



Scheme 1. (a) Formation of sulfenamide prodrug from an NH-acid drug; (b) bioreconverion of sulfenamide prodrug by glutathione (GSH) to form an NH-acid drug; (c) hydrolysis of a sulfenamide prodrug to form an NH-acid drug and a sulfenic acid.

Keywords: Prodrug; Sulfenamide; NH-acid; Amide; Bioavailability; Solubility; Dissolution rate; Permeability; Stability.
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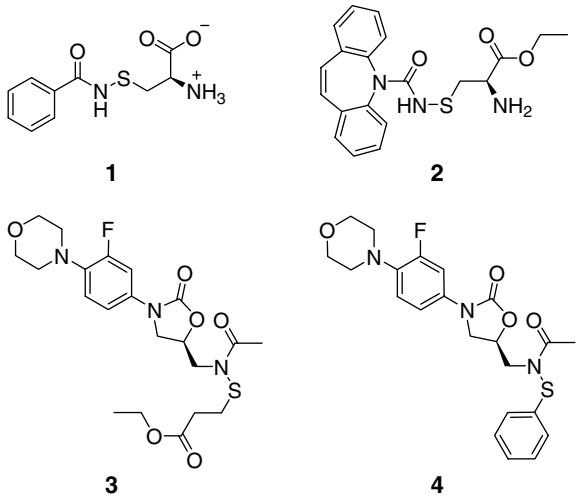
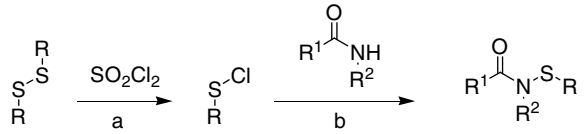
breaks the N–S single bond and forms an NH-acid product. Sulfenamides are known to rapidly react with free thiols, explaining their effectiveness as sulfenylating agents in the synthesis of asymmetrical disulfides.[7] Since free thiols are ubiquitous in vivo (cysteine, glutathione (GSH), etc.), a sulfenamide prodrug should conceivably regenerate the parent NH-acid upon introduction into the blood, as shown in Scheme 1b. Because the R-group in the promoiety is not explicitly defined, this approach would allow introduction of either hydrophilicity or lipophilicity to the parent drug, depending on the properties of the chosen R-group, allowing great flexibility in prodrug design. Furthermore, the attachment of the promoiety temporarily removes a hydrogen bond donor and adds steric bulk via the promoiety, both of which could increase the solubility through disruption of crystal packing interactions in the solid state.[8] Therefore with the appropriate promoiety, it is possible to increase the lipophilicity and the aqueous solubility simultaneously, which could have a dramatic impact on oral bioavailability.

To evaluate whether both hydrophilic and lipophilic sulfenamides of various weakly acidic NH-acids can behave as prodrugs, **1**, **2**, and **3**–**4** were synthesized as model sulfenamide prodrugs of benzamide (amide; model compound), carbamazepine (urea; drug example), and linezolid (amide; drug example), respectively, and were evaluated for aqueous chemical stability and reconversion kinetics.[5,9] To our knowledge, this work[5] represents the first formal aqueous pH-stability characterization for sulfenamides. Procedures for making sulfenamides have been established in the synthetic literature.[6] The general synthetic pathway utilized for **1**–**4** is shown in Scheme 2. In this approach, the appropriate disulfides were converted to sulfenyl chlorides in situ through reaction with sulfuryl chloride, and these sulfenyl chlorides were further reacted, without purification, with either the free NH-acid (in the presence of triethylamine), or the salt of the NH-acid, to form the sulfenamide product. Although this is a standard synthetic procedure for making sulfenamides, there have been procedural modifications using thiophthalimides instead of sulfenyl halides that, while not used in this work, have resulted in cleaner reactions and higher yields.[10]





Scheme 2. Generic synthetic pathway for sulfenamides. Reagents and conditions: (a) THF, 0 °C for 10 min; (b) TEA, THF, 0 °C for 20 min, rt for 60 min. (*Note.* TEA not needed if salt of NH-acid is used.)

The immediate hydrolysis products of a sulfenamide are expected to be the corresponding NH-acid and sulfenic acid components (Scheme 1c). Due to the general instability of sulfenic acids,[11] the N–S hydrolysis was followed by monitoring the loss of sulfenamide and the formation of the corresponding NH-acid, using HPLC/UV. Due to good hydrolytic stability, the pH-rate profile for degradation of **1** to form benzamide was determined at 50, 60, and 70 °C.[5] At a given pH and temperature, the hydrolysis of **1** followed pseudo-first-order kinetics. The pH-rate profile for **1** at 25 °C (Fig. 1) was projected by extrapolation from the elevated temperature results using the Eyring equation, and is shown as a U-shaped profile with a maximum hydrolytic stability at ~pH 6. Assuming the zwitterionic form of **1** was the predominant species degrading over the pH range studied, the projected points in Fig. 1 were fit[12] to the following kinetic model (Eq. 1), which is a summation of acid-catalyzed ($k_H[H^+]$), base-catalyzed ($k_{OH}K_w/[H^+]$), and uncatalyzed (k_o) hydrolytic mechanisms ($K_w = 1.0 \times 10^{-14}$ at 25 °C). From this mathematical fit, values were obtained for k_H ($5.37(\pm 0.12) \times 10^{-2}$ min^{-1} M^{-1}), k_o ($3.38(\pm 1.99) \times 10^{-8}$ min^{-1}) and

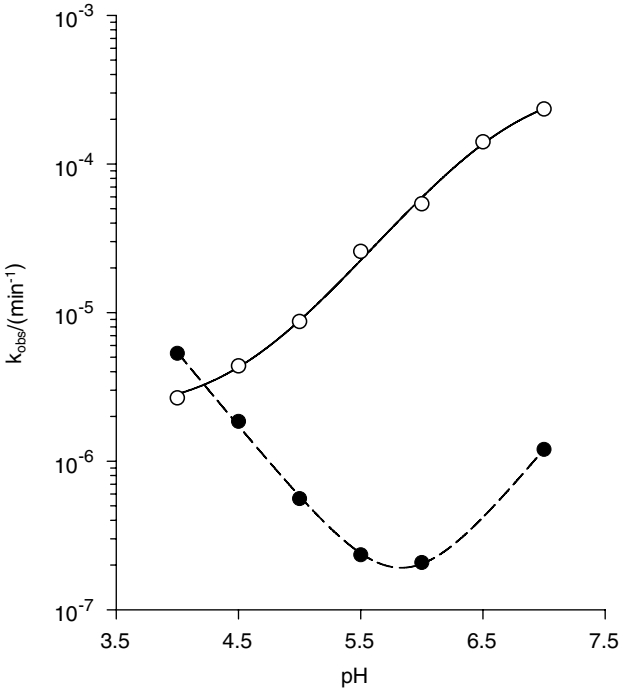


Figure 1. pH-rate profiles of **1** (●) and **2** (○) at 25 °C in 35 mM buffered solutions (ionic strength of 0.15 using NaCl). The dashed and solid lines represent mathematical fits for **1** (Eq. 1) and **2** (Eq. 5), respectively.

k_{OH} (11.6(±0.7) min^{-1} M^{-1}). Compound **1** is a very stable sulfenamide with regard to hydrolysis, as shown by its maximum projected half-life of ~6.3 years at pH 6.0 and 25 °C (Table 1). However, a dramatic acceleration in N–S degradation was seen when cysteine was added into a 23.3 μM, pH 5.5, solution of **1**, where the final cysteine (Cys) concentration was 233 μM, a 10-times excess[13] relative to **1**. Because the observed pseudo-first order degradation rate constant in the presence of Cys ($k^*_{obs} = 1.39 \times 10^{-2}$ min^{-1}) was 4–5 orders of magnitude greater than the projected hydrolysis rate constant in the absence of Cys ($k_{obs} = 2.34 \times 10^{-7}$ min^{-1}), the Cys-based degradation profile could be mathematically fit according to Eq. (2), allowing for a calculation of k_{cys} (59.7 min^{-1} M^{-1}) at pH 5.5 and 25 °C.

$$k_{obs} = k_H[H^+] + k_o + \frac{k_{OH}K_w}{[H^+]} \quad (1)$$

$$k^*_{obs} = k_{cys}[Cys] \quad (2)$$

Although not tested, it is probable that the assumed Cys-based thiolysis of **1** is driven mainly by the thiolate fraction present in solution, and therefore would be expected to occur even faster at pH 7.4 since the concentration of thiolate in solution would be ~1–2 orders of magnitude greater than at pH 5.5. Based on these results, **1** certainly possesses the desired duality of good chemical stability for formulation purposes plus fast reconversion kinetics to form the parent in the presence of a thiol (cysteine in this case), and therefore supports the notion that sulfenamides can behave as successful prodrugs of weakly acidic NH-acids.

Compound **2** was also evaluated for aqueous stability over the same pH range as **1** (Fig. 1).[5] It is important to note that the promoiety in **2** is the ethyl ester version of the cysteinyl promoiety in **1**. For a given non-ionizable NH-acid, it is conceivable that this esterified cysteinyl promoiety would lend improved dissolution rate and solubility properties to the prodrug, relative to the non-esterified cysteinyl promoiety found in **1**, since the latter would be predominantly in the zwitterionic state across the great majority of the physiologically relevant pH range. The degradation of **2** was followed at 25 °C for pH 6.0–7.0 and at elevated temperatures for pH 4.0–5.5, followed by subsequent projection to 25 °C using the Eyring equation. For most of the pH range studied, the predominant hydrolysis reaction for **2** was actually ethyl ester hydrolysis in the promoiety (determined by LC/MS), not N–S bond hydrolysis. Only at the very low end of the pH range did the ethyl ester become relatively stable enough such that N–S bond hydrolysis became the predominant reaction. Over the pH range studied, it was assumed that the protonated form of **2** was the predominant species undergoing degradation, and a kinetic model (Eq. 3) was developed describing two basic reactions: a base-catalyzed ethyl ester hydrolysis ($k_{OEt}K_w/[H^+]$) and an uncatalyzed sulfenamide hydrolysis (k_{NS}). The fractional term (f_+), describing the protonated amount of **2** (Eq. 4), can be substituted into Eq. (3) to get Eq. (5), which is a more explicit form of Eq. (3) containing the acid dissociation constant for the amine conjugate acid (K_a). Using Eq. (5) to fit[12] the pH-rate profile for **2**, values for k_{OEt} (6.85(±0.49) × 10^3 $M^{-1}min^{-1}$), k_{NS} (2.15(±0.67) × 10^{-6} min^{-1}), and K_a (1.92(±0.27) × 10^{-7}) were calculated.

$$k_{obs} = f_+ \times \left(k_{NS} + \frac{k_{OEt}K_w}{[H^+]}\right) \quad (3)$$

$$f_+ = \frac{[H^+]}{[H^+] + K_a} \quad (4)$$

$$k_{obs} = \frac{k_{NS}[H^+] + k_{OEt}K_w}{[H^+] + K_a} \quad (5)$$

Given the ethyl ester side reaction that was predominant over the majority of the pH range, **2** is certainly not the ideal model compound to directly evaluate N–S hydrolytic stability. But it can be indirectly gathered that over the majority of the pH range studied, the N–S bond was at least more stable than the ethyl ester functionality in the promoiety; furthermore, at pH 4.0, where the degradation was predominantly driven by N–S hydrolysis, the half-life of **2** was projected (from elevated temperature studies) to be ~180.7 days at 25 °C (Table 1), which is very good aqueous stability for most formulation purposes, thereby providing a second example that sulfenamides designed from weakly acidic NH-acids can exhibit sufficient hydrolytic stability for formulation purposes. Furthermore, due to the flexibility inherent in the sulfenamide approach, it should be fairly straightforward to design a more hydrolytically stable carbamazepine-based sulfenamide by choosing a promoiety that does not contain labile groups such as the ethyl ester in **2**.

Finally, the two linezolid-based sulfenamide prodrugs, **3**–**4**, were evaluated for reconversion kinetics in dog whole blood.[5] While a formal pH-stability study was not conducted on these sulfenamides, they were certainly stable enough to conduct, without significant hydrolytic interference, both whole blood reconversion experiments and MDCK (Madin–Darby canine kidney) cell transport permeability studies,[5] the latter of which is not described in this communication. When spiked into 37 °C pre-heated Beagle dog whole blood to form an

Table 1. Projected and actual observed hydrolysis rate constants and half-lives for compounds **1** and **2** at 25 °C

pH	Compound **1**		Compound **2**	
	k_{obs} ($min^{-1} \times 10^{-6}$)	$t_{1/2}$ (yr)	k_{obs} ($min^{-1} \times 10^{-5}$)	$t_{1/2}$ (day)
4.0	5.32	0.25	0.266	180.7
4.5	1.85	0.71	0.437	110.2
5.0	0.559	2.36	0.870	55.4
5.5	0.234	5.64	2.58	18.7
6.0	0.208	6.33	5.40[a]	8.9[a]
6.5	—	—	14.1[a]	3.4[a]
7.0	1.20	1.10	23.4[a]	2.1[a]

[a] Actual 25 °C data. All other data in table is projected from elevated temperatures.

initial concentration of ~40 μM, compounds **3** and **4** yielded equimolar amounts of linezolid in essentially an *instantaneous* and *quantitative* fashion. In fact, the conversion to linezolid was complete for both **3** and **4** before the first spiked blood sample could be sufficiently mixed and subsequently quenched (<2 min).[14] This rapid and quantitative reconversion is highly desired for the vast majority of prodrug uses, and provides strong support for these compounds performing as prodrugs in vivo, particularly since the in vivo reconversion for sulfenamide prodrugs is expected to be chemically driven by reaction with thiols, not enzymatic in nature.

While there remains the need to evaluate the pharmacokinetics and toxicology of some selected sulfenamide prodrugs, some of which is being explored, the results presented in this communication provide strong support for sufenamides successfully behaving as prodrugs of weakly acidic NH-acids. As mentioned previously, there is a significant void in the synthetic literature for designing prodrugs of weakly acidic NH-acids, and it appears as if sulfenamides could fill this current need in the medicinal chemist's portfolio of prodrug technologies.

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9. Compound **1**: ^{1}H NMR (DMSO-d_6) δ 3.00–3.12 (m, 1H), 3.20–3.28 (m, 1H), 3.40–4.20 (br), 4.07–4.20 (br m, 1H), 7.46 (t, 2H), 7.53 (t, 1H), 7.80 (d, 2H), 8.30–8.50 (br), 9.65 (s, 1H); HRMS (FAB+, PEG300) *m/z* calcd for $C_{10}H_{13}N_2O_3S$ $(M+H)^+$ 241.0647, found 241.0643.Compound **2**: ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H), 2.83 (m, 1H), 3.05 (m, 1H), 4.02 (br m, 1H), 4.15 (q, 2H), 6.95 (s, 2H), 7.08 (s, 1H), 7.33 (m, 2H), 7.41 (br m, 6H), 8.40 (br s); HRMS (FAB+, PEG300) *m/z* calcd for $C_{20}H_{22}N_3O_3S$ $(M+H)^+$ 384.1382, found 384.1369.Compound **3**: ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H) 2.35 (s, 3H), 2.62 (t, 2H), 3.00 (m, 4H), 3.13 (m, 2H), 3.63 (dd, 1H), 3.67 (dd, 1H), 3.82 (m, 4H), 3.98 (t, 1H), 4.07 (m, 1H), 4.14 (q, 2H) 4.86 (m, 1H), 6.88 (t, 1H), 7.05 (dd, 1H), 7.40 (dd, 1H); HRMS (FAB+, PEG600) *m/z* calcd for $C_{21}H_{28}F_1N_3O_6S$ $(M)^+$ 469.1683, found 469.1684. Compound **4**: ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H), 3.00 (m, 4H), 3.70 (dd, 1H), 3.83 (m, 4H), 3.88 (dd, 1H), 3.98 (t, 1H), 4.07 (dd, 1H), 4.88 (m, 1H), 6.88 (t, 1H), 7.07 (t, 3H), 7.22 (t, 1H), 7.35 (m, 3H); HRMS (ES+, 99% methanol, 5mM ammonium acetate) *m/z* calcd for $C_{22}H_{25}F_1N_3O_4S$ $(M+H)^+$ 446.1550, found 446.1545.
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12. SigmaPlot 2004 version 9.01. Fit k_{obs} vs pH with a weighting factor of $1/k_{obs}$.
13. A 10 times excess of cysteine was used to create pseudo-first order degradation conditions for **1** allowing a straightforward calculation of k_{cys}.
14. *Beagle dog whole-blood experiment.* For a given compound (**3**, **4** or linezolid), 15 μL of an 8 mM DMSO stock solution was added to 3 mL blood (37 °C) and vortexed for 5 s. A 400 μL aliquot of spiked blood was mixed with 400 μL chilled acetonitrile and 100 μL chilled saturated aqueous $(NH_4)_2SO_4$ solution. The mixture was vortexed for 5 s and centrifuged for 30 s. The supernatant was collected and immediately analyzed by HPLC. Control experiments showed that **3**, **4**, and linezolid were chemically stable in the supernatant fraction over the time course of the sample preparation and analysis.